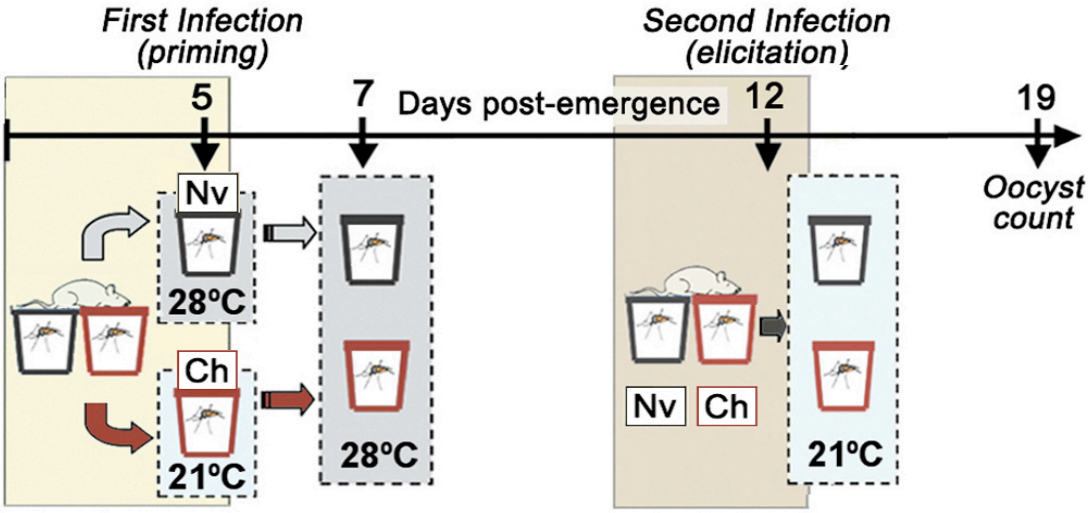

Fig. S1. Effect of pre-exposure to *Plasmodium* infection on the immune response to subsequent infections. Schematic representation of the experimental design. The timeline depicts the mosquito age in days. Shaded areas represent the duration of antibiotic treatments before the first (yellow area) or second (beige area) infection. Effect of pre-challenge with *Plasmodium berghei* on a second infection with the same parasite.

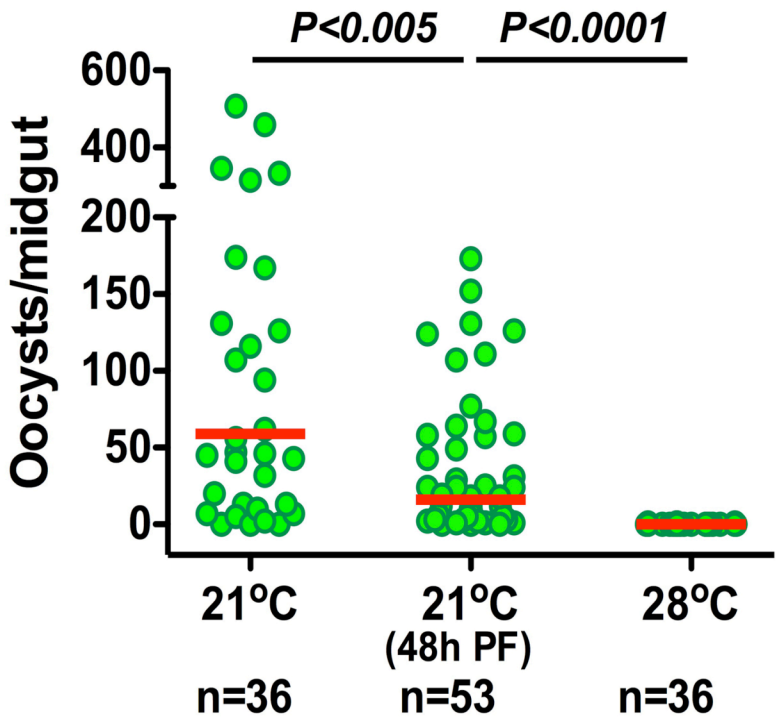

Fig. S2. Effect of temperature on *Plasmodium berghei* infection in *Anopheles gambiae* mosquitoes. Three groups of mosquitoes were fed on the same *P. berghei*-infected mouse. After feeding, mosquitoes were either kept at 21°C (a permissive temperature for *P. berghei* infection), or kept at 21°C for 48 hours to allow ookinete development and midgut invasion and then transferred to 28°C (a non-permissive temperature) to reduce *P. berghei* infection. A third group was placed at 28°C immediately after feeding to prevent ookinete formation and mosquito infection. *Plasmodium* infection was evaluated 7 days after infection. Each circle represents the number of oocysts on an individual midgut; the line indicates the median. Distributions were compared using the Mann-Whitney test.

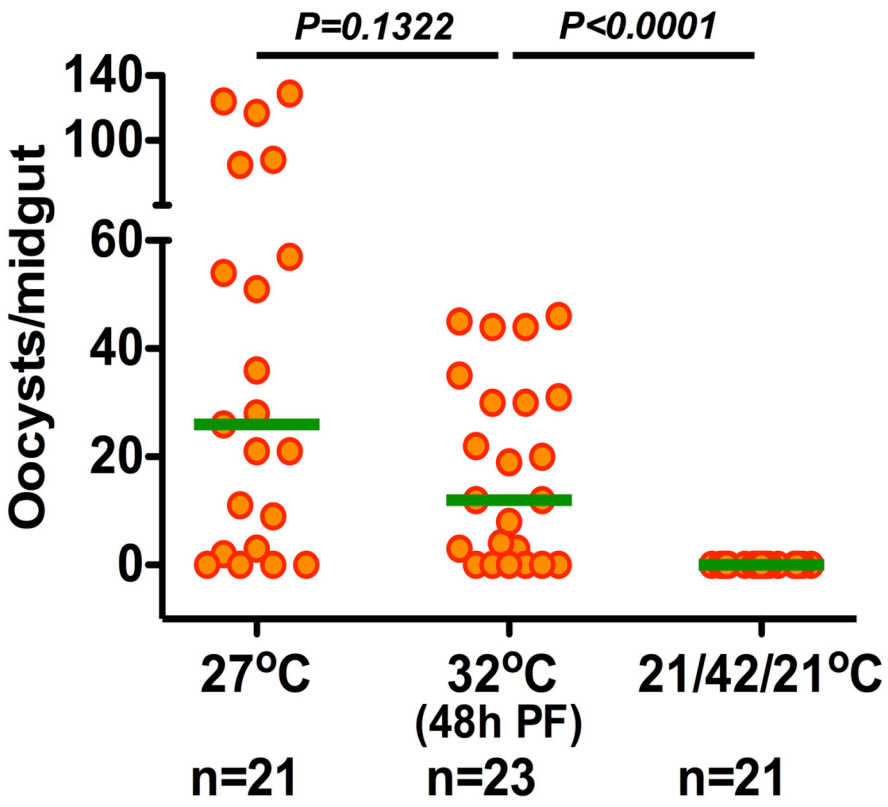

Fig. S3. Effect of temperature on *Plasmodium falciparum* infection. Three groups of mosquitoes were fed a *P. falciparum* NF54 gametocyte culture and either kept at 27°C, a permissive temperature for *P. falciparum*, or kept at 27°C for 48 hours to allow for ookinete midgut invasion and then transferred to 32°C to reduce the oocyst burden. A third group was fed on the same gametocyte culture, which had been inactivated by incubating it at 21°C (30 min)/42°C (30 min)/21°C (30 min); and was also kept at 27°C for 48 hours after feeding and then switched to 32°C. Each circle represents the number of oocysts on an individual midgut, and the line indicates the median. Distributions were compared using the Mann-Whitney test.

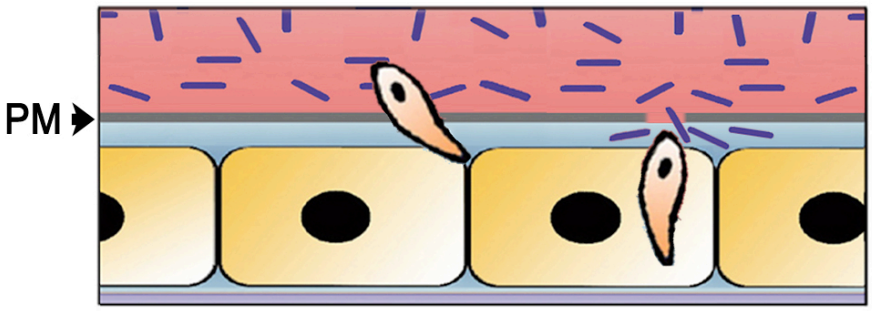

Fig. S4. Model of the effect of ookinete midgut invasion on the physiological midgut barriers. Ookinetes digest their way through the peritrophic matrix (PM) and invade midgut epithelial cells. During this process, parasites disrupt the gut barriers and allow bacteria from the microbiota (blue rods) to come in direct contact with injured midgut cells.

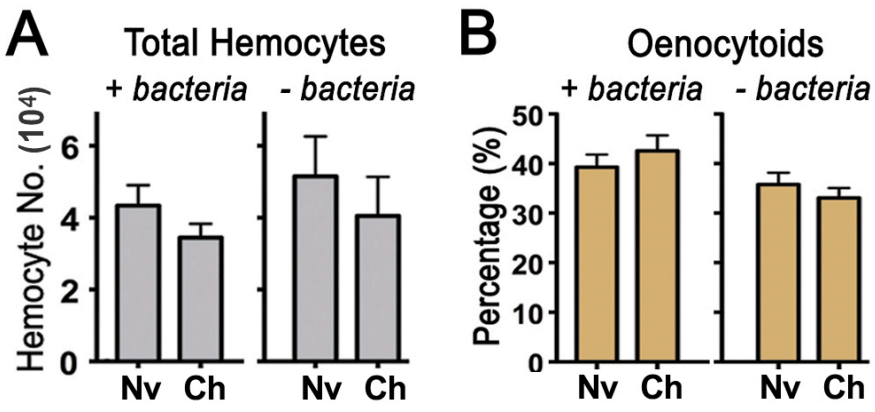

Fig. S5. Effect of immune priming on (**A**) the total number of circulating hemocytes and on (**B**) the relative abundance of oenocytoids 7 days post priming in the presence (+ bacteria) or absence (– bacteria) of gut microbiota (Mean ± SEM).

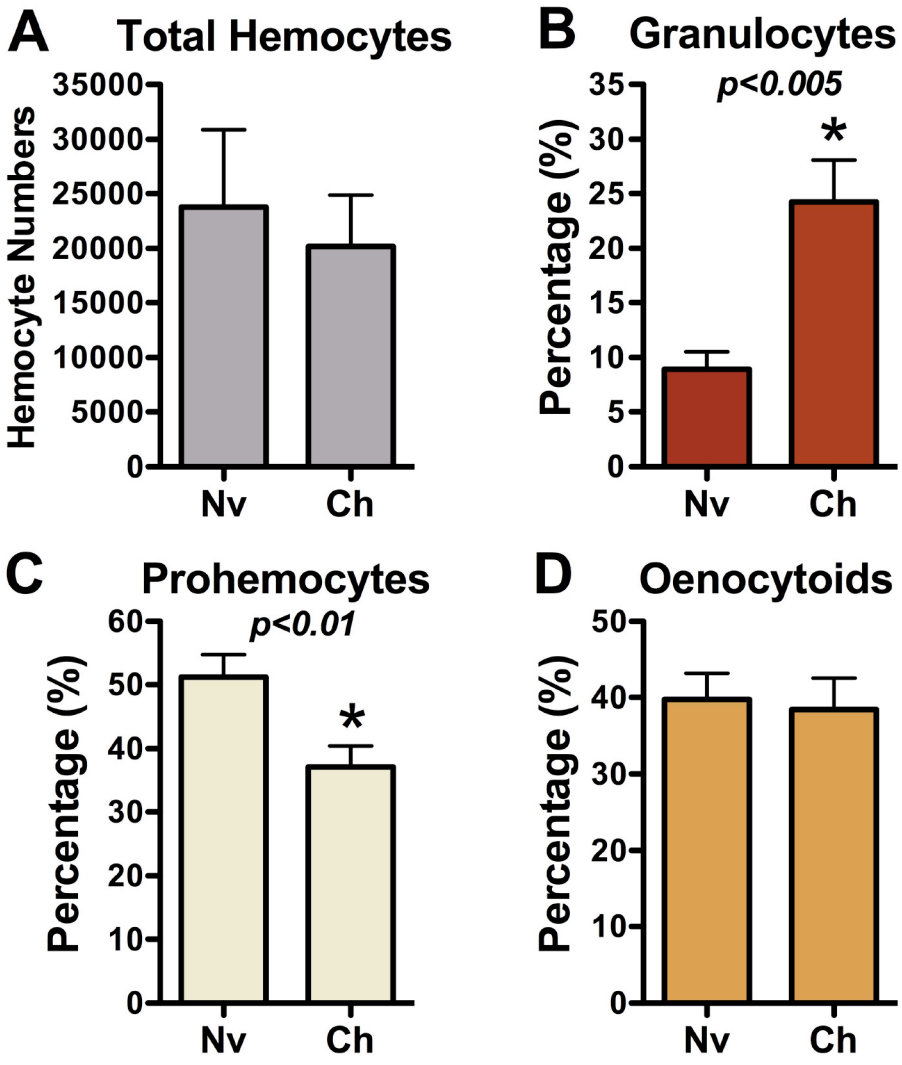

Fig. S6. Effect of immune priming on the total number of circulating hemocytes (**A**) and on relative abundance of granulocytes (**B**), prohemocytes (**C**), or oenocytoids (**D**) 14 days after priming (Mean ± SEM).

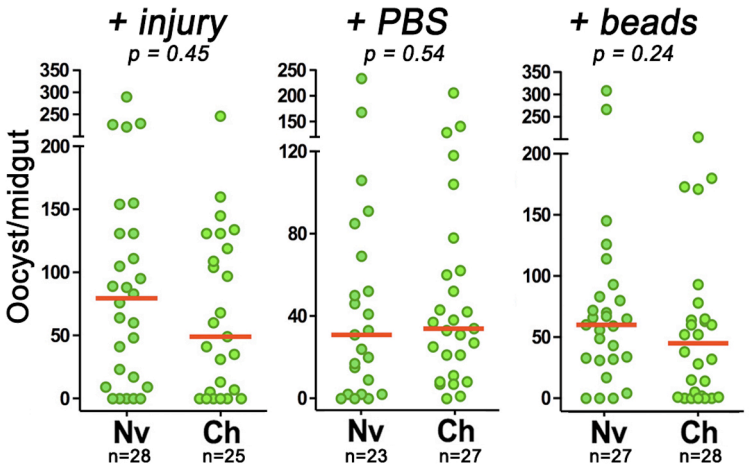

Fig. S7. Effect of aseptic injury, injection of PBS or Sephadex beads on priming of antiplasmodial responses. Naïve (Nv) and challenged (Ch) mosquitoes were subjected to sterile injury, injected with PBS or Sephadex beads 7 days post priming, and infected with *Plasmodium berghei* two days later. *Plasmodium* infection was evaluated 7 days post infection. Each circle represents the number of oocysts on an individual midgut, and the line indicates the median. Distributions were compared using the Mann-Whitney test.

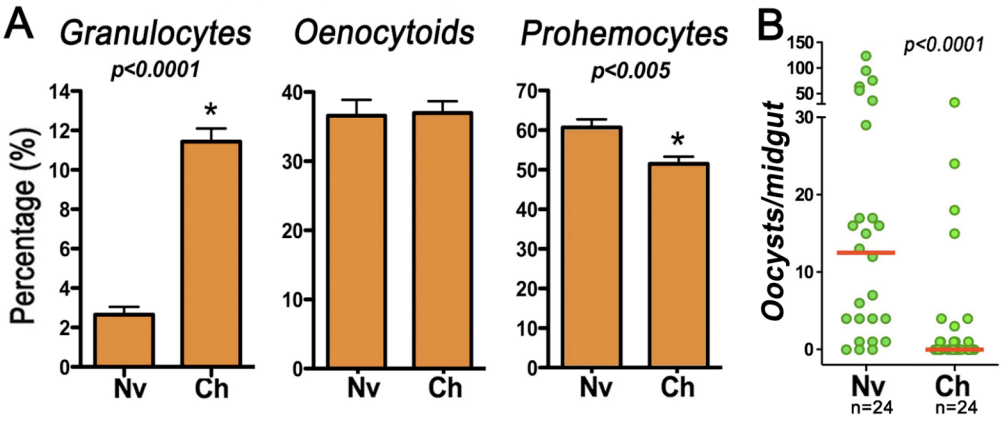

Fig. S8. Effect of transferring hemolymph containing hemocytes from naïve (Nv) or challenged (Ch) donors into recipient mosquitoes. (**A**) Effect on the relative abundance of granulocytes, oenocytoids, and prohemocytes four days post transfer (Mean ± SEM). (**B**) Effect of transfer on *Plasmodium berghei* infection. *Plasmodium* infection was evaluated 7 days after infection. Each circle represents the number of oocysts on an individual midgut ,and the line indicates the median. Distributions were compared using the Mann-Whitney test.

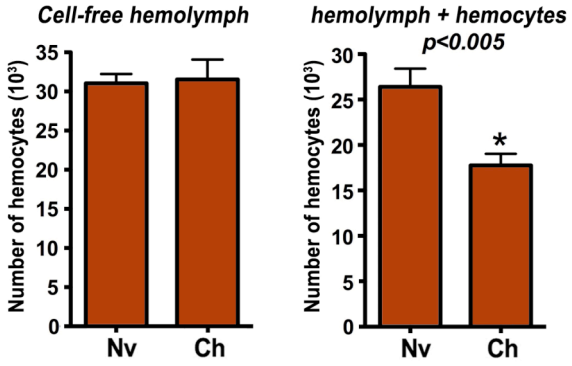

Fig. S9. Effect of transfer of (**A**) cell-free hemolymph and (**B**) hemolymph containing hemocytes from naïve (Nv) or challenged (Ch) donor mosquitoes on the number of circulating hemocytes in recipients four days post transfer (Mean ± SEM).